RWLP0496

B999 QJ259 190
EPIDEMIOLOGY 95
(C)WILLIAMS & WILKINS MD

23648

COMMENTARY

# Limitations of Biomarkers of Exposure in Cancer Epidemiology

*Neil Pearce,*[1,2] *Silvia de Sanjose,*[2] *Paolo Boffetta,*[2] *Manolis Kogevinas,*[2] *Rodolfo Saracci,*[2] *and David Savitz*[3]

In recent years, there has been increasing interest in the use of biomarkers of exposure in cancer epidemiology. In addition, markers of early stages of the disease process, or of individual susceptibility, are expected to provide new insights into the mechanisms of cancer causation.[1] In particular, there has been an explosion of interest in "molecular cancer epidemiology," which has been defined as:

"an approach in which advanced laboratory methods are used in combination with analytical epidemiology to identify at the biochemical or molecular level specific exogenous agents and/or host factors that play a role in human cancer causation."[2]

Despite the cautious conclusion of some reviewers[3] that molecular epidemiology is a field that has shown significant promise but only limited success to date, others have argued that:

"We are in the era of molecular research. . . . Progress in the molecular approach to biology and medicine has stimulated and excited both the public and researchers, who now believe that these advances can be applied to the study, prevention, and control of health risks faced by human populations. . . . The use of molecular markers represents a quantum leap in the evolution of epidemiologic ideas."[1]

The term "molecular epidemiology" is itself questionable. Branches of epidemiology are usually defined in substantive terms describing the types of exposure (for example, occupational epidemiology) or disease (for example, cancer epidemiology) or the groups of subjects under study (for example, pediatric epidemiology). It is unusual to define a branch of epidemiology in terms of a particular set of techniques (for example, no one uses the terms "statistical epidemiology" or "questionnaire epidemiology"); to do so places emphasis on the techniques, rather than on the hypotheses to be investigated.

In this paper, we will consider biomarkers of exposure as a broad group, but we will focus on the newly developed molecular markers of exposure and compare them with more traditional methods of exposure assessment. We will not discuss biomarkers of early disease and susceptibility,[4] which involve very different scientific and public health issues. This paper is not intended to be a comprehensive review; rather, our intention is to discuss the general issues involved in using biomarkers and to place them into perspective. In particular, we will argue that there are considerable scientific limitations to the use of biomarkers of exposure in cancer epidemiology studies, that the current overemphasis on this approach is misplaced, and that biomarkers have both strengths and limitations relative to other methods of exposure assessment. Some of these limitations apply to the biomarkers currently available and may be overcome with further technologic developments, but there are many situations in which the use of biomarkers is inappropriate even in principle.

We will concentrate on the situation where a single exposure-disease relation is under study, although the exposure in question may be a complex mixture (such as tobacco smoke). This approach does not consider the interdependence of multiple agents, or more general issues, such as how human populations become exposed and susceptible,[5] or the role of general improvements in nutrition, housing, and income.[6] Nevertheless, there is an important role for studies of specific agents, in both scientific and public health terms, and it is in this area that biomarkers have the greatest potential.

## Successful Uses of Biomarkers of Exposure

Biomarkers of exposure are neither new nor lacking a record of considerable success in epidemiology. For example, liver function assays, urinary phenol levels, and blood lead levels have long been used in occupational epidemiology.[7] Similarly, markers of viral exposures have played a major role in many epidemiologic discoveries. A recent example is the use of a technique (the polymerase chain reaction) for the identification of human

From the [1]Department of Medicine, Wellington School of Medicine, Wellington, New Zealand; [2]International Agency for Research on Cancer, Lyon, France; and [3]Department of Epidemiology, University of North Carolina, Chapel Hill, NC.

Address correspondence to: Neil Pearce, Wellington School of Medicine, P.O. Box 7343, Wellington, New Zealand.

This work was conducted in part during the tenure by Neil Pearce of a Senior Research Fellowship of the Health Research Council of New Zealand, and in part during the tenure of a Visiting Scientist Award of the International Agency for Research on Cancer.

Submitted October 12, 1993; final version accepted July 5, 1994.

© 1995 by Epidemiology Resources Inc.



THIS ARTICLE IS FOR INDIVIDUAL USE ONLY AND MAY NOT BE FURTHER REPRODUCED OR STORED ELECTRONICALLY WITHOUT WRITTEN PERMISSION FROM THE COPYRIGHT HOLDER. UNAUTHORIZED REPRODUCTION MAY RESULT IN FINANCIAL AND OTHER PENALTIES.

PM3006509375

Source: https://www.industrydocuments.ucsf.edu/docs/stgj0001

Papillomavirus deoxyribonucleic acid (DNA), which provided strong evidence of a more important role of this virus in the etiology of human cervical cancer than had previously been recognized.[8] Similar successes have been achieved with other oncogenic viruses, such as the hepatitis B virus.[9]

Another successful use of biomarkers of exposure in cancer epidemiology relates to the association between dietary exposure to aflatoxins and liver cancer risk.[10] This association had been recognized for nearly 20 years,[11] but until recently, almost all of the epidemiologic studies were ecologic or were based on relatively poor exposure information, such as consumption of possibly contaminated food. Biomarkers of exposure to aflatoxins were shown recently to be associated with elevated liver cancer risk.[12] Although these markers only reflect recent exposures, it is likely that they provide a better measure of historical aflatoxin exposure than is provided by personal interviews or market surveys of contaminated food stocks,[13] since the level of contamination varies greatly, owing to temporal and local factors.

Thus, situations in which molecular biology techniques have enhanced the identification and quantification of important risk factors for human cancer primarily involve exposure to viruses or other biological agents. In other contexts, the historical evidence for the value of biomarkers is less encouraging. Most major causes of cancer have been discovered and quantified on the basis of questionnaires (for example, tobacco smoking) or questionnaires combined with environmental measurements (for example, asbestos).

## Current Limitations of Biomarkers of Exposure

### Historical Exposures

Perhaps the major limitation of biomarkers is that they usually only indicate relatively recent exposures. For example, it is well known that serum levels of micronutrients reflect recent rather than historical dietary intake.[14] For many cases of cancer, exposures that occurred 10–30 years previously are etiologically relevant. Some biomarkers are better than others in this respect (particularly biomarkers for exposure to biological agents), but even the best markers of chemical exposures reflect only the last few weeks or months of exposure. For example, a marker such as thiocyanate, which has a half-life of up to 14 days, is regarded as a marker of "long-term" exposure.[15] In the case of DNA adducts, Wilcosky and Griffith[16] argue that:

> "a typical case-control study of cancer and chemical exposures could not rely on this type of biological marker. The relevant exposures occur many years before disease diagnosis, and any DNA adducts from the relevant exposure period will probably have disappeared or be indistinguishable from adducts formed more recently."

On the other hand, with some biomarkers (for example, serum levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin[17]), it may be possible to estimate historical levels if the exposure period is known, the half-life is relatively long (and is known), and it is assumed that no significant exposure has occurred more recently, or if it is reasonable to assume that exposure levels have remained stable over time.

Nevertheless, historical information on exposure surrogates will often be more valid than current direct measurements of exposure or dose. This situation has long been recognized in occupational epidemiology, where the use of work history records in combination with a job-exposure matrix (based on historical exposure measurements of work areas rather than individuals) usually is more valid than current exposure measurements (whether based on environmental measurements or biomarkers) because of changes in exposure levels over time.[18] Similar problems may occur in the measurement of other carcinogenic exposures. For example, even the best currently available measures of exposure to tobacco smoke, such as plasma or urinary cotinine, appear to have similar validity to questionnaires for the measurement of current exposures[19–21]; their very short half-life makes them inferior to questionnaires in the estimation of historical exposures. On the other hand, some biomarkers would appear to have value in the validation of questionnaires,[22] which can then be used to measure historical exposures.

### Individual Temporal Variation

It might be hoped that these problems of using biomarkers to measure historical exposures can be minimized in cohort studies involving stored biological samples (perhaps to be used in nested case-control studies). Nevertheless, such biological samples may not validly measure long-term patterns of exposure when there are significant variations in exposure over time, unless samples have been taken repeatedly over the course of the study.[4]

### Study Size and Power

A further problem with the use of biomarkers in cancer epidemiology studies (and particularly in cohort studies) is that of small numbers. Even large multicentric cohort studies often struggle to obtain sufficient numbers to assess risks of rare cancers from occupational exposures.[23,24] The use of biomarkers may be a major problem in this regard, since the resulting expense and complexity may drastically reduce the study size, even in a case-control study, and therefore greatly reduce the statistical power for detecting an association between exposure and disease. The optimal balance between precision and validity depends on a number of considerations, including the relative costs of the various exposure measurement techniques.[25] Thus, for a biomarker to be useful, it must be substantially better than less expensive and less invasive approaches.

## Inherent Limitations of Biomarkers

Many of the current limitations of biomarkers in cancer epidemiology, particularly those relating to study size and historical exposures, will presumably be overcome as

PM3006509376

Source: https://www.industrydocuments.ucsf.edu/docs/stgj0001

more valid, convenient, and inexpensive biomarkers are developed. There are, however, more inherent limitations of biomarkers that will continue to restrict their applicability.

### What Does a Biomarker Measure?

One key problem with biomarkers is that they measure the internal dose (the "biologically effective dose") rather than the external exposure. This characteristic is often claimed to be their main benefit,[26] but for many biomarkers (for example, mutagenic activity in urine), it is not always clear whether one is measuring the exposure, the biological effect, or some stage of the disease process itself.[27] Thus, the findings may be uninterpretable in terms of the causal association between exposure and disease.

In fact, there are few clear examples that demonstrate that biomarkers will more clearly reflect the individual response to exposure,[28] and hence individual cancer risk. In this context, the most pertinent question is not whether a biomarker predicts disease, but whether it predicts it better than simpler methods of exposure assessment. For example, the presence of pleural plaques presumably reflects individual susceptibility or high cumulative exposure to asbestos, or both, but there is little clear evidence that the presence of pleural plaques is a better predictor of lung cancer risk than more traditional exposure measures based on the intensity and duration of exogenous asbestos exposures.[27]

When it is known that the "biologically effective dose" is the most appropriate measure, then the use of appropriate biomarkers clearly has some scientific advantages over other methods of exposure assessment. Choosing the appropriate biomarker is a major dilemma, however, and biomarkers are frequently chosen on the basis of an incomplete or erroneous understanding of the etiologic process, or simply because a particular marker can be measured. An environmental exposure (for example, tobacco smoke) may involve hundreds of different chemicals and may produce hundreds of measurable biological responses. A biomarker typically measures one of the biological responses to one of the chemicals. If the chosen biomarker measures the key etiologic factor, then it may yield relatively good exposure data; on the other hand, if a biomarker is chosen that has little relation to the carcinogenic component of the complex exposure mixture, then the biomarker will yield relatively poor exposure data. The potential for such problems is shown by the observation that, among individuals who smoke similar amounts of tobacco, the number of benzo(*a*)pyrene covalent binding sites to DNA may vary 100-fold across individuals.[29] Thus, very poor exposure data will be obtained if the wrong biomarker is chosen. For example, plasma cotinine appears to have a nonlinear relation to the number of cigarettes smoked per day,[30] which in turn is strongly predictive of lung cancer risk; thus, plasma cotinine may be a relatively poor quantitative marker of exposure to the constituents of cigarette smoke that are etiologically relevant to lung cancer.

An analogous example is the role of dietary micronutrients in the prevention of cancer. Numerous studies have shown that the consumption of green and yellow vegetables is protective against a range of cancers, but the identification of the specific dietary micronutrients involved has remained elusive.[31] Although it is likely that an antioxidant mechanism is involved, the various antioxidants appear to act in synergy,[29] and it is unclear whether epidemiologic studies will be able to identify the specific micronutrients involved, whether this would be useful in public health terms, or whether it is appropriate scientifically to view such a complex process in such reductionist terms.[5]

### Biomarkers May Introduce Confounding

A related problem is that biomarkers usually indicate the total exposure to a substance from all sources. This blending may be inappropriate for attempting to assess the effects of a particular environmental exposure. For example, measuring polycyclic aromatic hydrocarbon (PAH)-DNA adducts may be problematic in an occupational study, since PAH-DNA levels reflect exposures from all sources, including industrial pollution, fossil fuels, dietary exposures, and cigarette smoke, and are influenced by seasonal variations.[29] Such markers may be more susceptible to confounding than traditional measures,[7] since other risk factors for cancer may be more strongly associated with these additional routes of exposure than they are associated with the main exposure route of interest.

Consider a study of lung cancer and polycyclic aromatic hydrocarbon exposure in a group of factory workers. If the workers are classified according to polycyclic aromatic hydrocarbon exposure on the basis of industrial hygiene monitoring, then the percentage of smokers (and the mean number of cigarettes smoked per day) will usually be similar in the groups with low, medium, and high levels of occupational exposure to polycyclic aromatic hydrocarbons (since these groups are defined purely on environmental levels of polycyclic aromatic hydrocarbon exposure in various job categories and departments, and these exposures will usually be unrelated to cigarette smoking[32]). In this situation, cigarette smoking will not be a major confounder. If workers are classified according to PAH-DNA levels, however, then these levels will indicate total exposure to polycyclic aromatic hydrocarbons from all sources, including cigarette smoking. Thus, cigarette smokers will be more likely to be in the "high-polycyclic aromatic hydrocarbon exposure" group, and this group will therefore contain a higher proportion of smokers (and a higher mean number of cigarettes smoked per day) than the medium- or low-polycyclic aromatic hydrocarbon exposure groups (since some of the total polycyclic aromatic hydrocarbon exposure comes from cigarette smoke). The dose response will then be confounded by cigarette smoking, and the "high-polycyclic aromatic hydrocarbon exposure" group may show a higher lung cancer risk that is not due to polycyclic aromatic hydrocarbon exposure,

PM3006509377

Source: https://www.industrydocuments.ucsf.edu/docs/stgj0001

but is actually due to the other carcinogenic constituents of cigarette smoke.

### Application to Public Health Policy

Finally, it should be noted that the "biologically effective dose" is rarely of interest in terms of public health policy, since regulation is usually based on environmental exposure levels. There are exceptions to this, and there are some instances (for example, serum lead levels) in which regulations may be based on individual measurements (or occasionally even on individual susceptibility). But the practical difficulties and implicit dangers of this approach are also widely recognized; it usually is more appropriate and more feasible to regulate work place exposures (thus placing responsibility on the employer) rather than to regulate individual workers (who may suffer punitive measures and loss of employment).

## Conclusion

The current enthusiasm for molecular biology techniques in epidemiology is analogous to other technologic advances in medicine and public health in recent decades. For example, the development of increasingly sophisticated methods of statistical analysis in recent decades has brought about a revolution in the practice of epidemiology. The unnecessary use of sophisticated methods of data analysis has been questioned more recently, and the strengths and limitations of such methods have been placed into a more appropriate perspective,[33] although these voices have perhaps not been the predominant ones.

Cancer epidemiology is now experiencing a comparable revolution in methods of exposure assessment. Once again, it is important that the strengths and limitations of these new tools are recognized and placed into perspective and that the findings obtained from complex biomarkers of exposure are checked against those from more straightforward (and historically reliable) methods, including questionnaires, routine records, and environmental monitoring. For these reasons, although cancer epidemiologists will continue to use biomarkers, traditional methods of exposure assessment will also continue to play an important role, which will be complemented (but not replaced) by the cautious use of newer and more complex methods of exposure assessment. Otherwise, there is the constant danger that epidemiologic "problems" will be chosen to utilize molecular biology techniques, while more important public health problems are ignored.

## Acknowledgments

We wish to thank Juha Pekkanen and Lorenzo Tomatis for their comments on the draft manuscript.

## References

1. Schulte PA. A conceptual and historical framework for molecular epidemiology. In: Schulte P, Perera FP (eds). Molecular Epidemiology: Principles and Practices. New York: Academic Press, 1993;3–44.
2. Perera FP, Weinstein IB. Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. J Chron Dis 1982;35:581–600.
3. Hulka BS. Overview of biological markers. In: Hulka BS, Wilcosky TC, Griffith JD (eds). Biological Markers in Epidemiology. New York: Oxford University Press, 1990;3–15.
4. Armstrong BK, White E, Saracci R. Principles of Exposure Measurement in Epidemiology. New York: Oxford University Press, 1992.
5. Loomis D, Wing S. Is molecular epidemiology a germ theory for the end of the twentieth century? Int J Epidemiol 1991;19:1–3.
6. McKeown T. The Role of Medicine. London: Nuffield Provincial Hospitals Trust, 1976.
7. Schulte PA. Contribution of biological markers to occupational health. Am J Ind Med 1991;20:435–446.
8. Muñoz N, Bosch FX, Sanjose S, Izarzugaza I, Gili M, Viladiu P, Navarro C, Martos C, Ascunce N, González LC, Kaldor JM, Guerrero E, Lorincz A, Santamaria M, Alonso de Ruiz P, Aristizabal N, Shah K. The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Colombia and Spain. Int J Cancer 1992;52:743–749.
9. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus: a prospective study of 22,707 men in Taiwan. Lancet 1981;2:1129–1133.
10. Wild CP, Jansen LA, Cova L, Montesano R. Molecular dosimetry of aflatoxin exposure: contribution to understanding the multifactorial etiopathogenesis of primary hepatocellular carcinoma with particular reference to hepatitis B virus. Environ Health Perspect 1993;99:115–122.
11. Alpert ME, Hutt MSR, Wogan GN, Davidson CS. Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer 1971;28:253–260.
12. Ross RK, Yuan J-M, Yu MC, Wogan GN, Qian G-S, Tu J-T, Groopman JD, Gao Y-T, Henderson BE. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet 1992;339:943–946.
13. Qian G-S, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD. A follow-up study of urinary markers of aflatoxin exposure and liver cancer in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers & Prev 1994;3:3–10.
14. Willett W. Nutritional Epidemiology. New York: Oxford University Press, 1990.
15. Sorsa M, Husgafvel-Pursiainen K. Assessment of passive and transplacental exposure to tobacco smoke. In: Bartsch H, Hemminki K, O'Neill IK (eds). Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention. Lyon: International Agency for Research on Cancer, 1988;129–32.
16. Wilcosky TC, Griffith JD. Applications of biological markers. In: Hulka BS, Wilcosky TC, Griffith JD (eds). Biological Markers in Epidemiology. New York: Oxford University Press, 1990;16–27.
17. Sweeney MH, Fingerhut MA, Patterson DG Jr, Connally LB, Piacitelli LA, Morris JA, Greife AL, Hornung RW, Marlow DA, Dugl JE, Halperin WE, Needham LL. Comparison of serum levels of 2,3,7,8-TCDD in TCP production workers and in an unexposed comparison group. Chemosphere 1990;20:993–1000.
18. Checkoway H, Pearce N, Crawford-Brown D. Research Methods in Occupational Epidemiology. New York: Oxford University Press, 1989.
19. Chilmonczyk BA, Salmun LM, Megathlin KN, Leveux LM, Palomaki GE, Knight GJ, Pulkkinen AJ, Haddow JE. Association between exposure to environmental tobacco smoke and exacerbations of asthma in children. N Engl J Med 1993;328:1665–1669.
20. Hulka BS. Methodologic issues in molecular epidemiology. In: Hulka BS, Wilcosky TC, Griffith JD (eds). Biological Markers in Epidemiology. New York: Oxford University Press, 1990;214–226.
21. Vineis P. Uses of biochemical and biological markers in occupational epidemiology. Rev Epidemiol Santé Publique 1992;40:S63–S69.
22. Forastiere F, Agabiti N, Dell'orco V, Pistelli R, Corbo GM, Brancato G, Pacifici R, Zuccaro P, Perucci CA. Questionnaire data as predictors of urinary cotinine levels among nonsmoking adolescents. Arch Environ Health 1993;48:230–234.
23. Saracci R, Kogevinas M, Bertazzi P, Bueno de Mesquita BH, Coggon D, Green LM, Kauppinnen T, L'Abbé KA, Littorin M, Lynge E, Mathews JD, Neuberger M, Osman J, Pearce NE, Winkelmann R. Cancer mortality in an international cohort of workers exposed to chlorophenoxy herbicides and chlorophenols. Lancet 1991;338:1027–1032.
24. Fingerhut MA, Halperin WE, Marlow DA, Piacitelli LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. N Engl J Med 1991;324:212–218.
25. Greenland S. Statistical uncertainty due to misclassification: implications for validation substudies. J Clin Epidemiol 1988;41:1167–1174.
26. Shields PG, Harris CC. Molecular epidemiology and the genetics of environmental cancer. JAMA 1991;266:681–687.
27. Saracci R. Assessing exposure of individuals in the identification of disease determinants. In: Berlin A, Draper M, Hemminki K, Vainio H (eds).

PM3006509378

Source: https://www.industrydocuments.ucsf.edu/docs/stgj0001

Monitoring Human Exposure to Carcinogenic and Mutagenic Agents. Lyon: International Agency for Research on Cancer, 1984.
28. Perera FP, Santella R. Carcinogenesis. In: Schulte P, Perera FP (eds). Molecular Epidemiology: Principles and Practices. New York: Academic Press, 1993;277–300.
29. Correa P. Molecular and biochemical methods in cancer epidemiology and prevention: the path between the laboratory and the population. Cancer Epidemiol Biomarkers & Prev 1993;2:85–88.
30. Pasingham BJ, Farmer PB, Bailey E, Brooks AGF, Yates DW. 2-Hydroxyethylation of haemoglobin in man. In: Bartsch H, Hemminki K, O'Neill IK (eds). Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention. Lyon: International Agency for Research on Cancer, 1988;279–285.
31. Steinmetz KA, Potter JD. Vegetables, fruit and cancer. I. Epidemiology. Cancer Causes Control 1991;2:325–357.
32. Siemiatycki J, Wacholder S, Dewar R, Wald L, Bégin D, Richardson L, Rosenman K, Gérin M. Smoking and degree of occupational exposure: are internal analyses in cohort studies likely to be confounded by smoking status? Am J Ind Med 1988;13:59–69.
33. Rothman KJ. Modern Epidemiology. Boston: Little, Brown, 1986.

PM3006509379

Source: https://www.industrydocuments.ucsf.edu/docs/stgj0001